

Genzyme Corporation (formerly GelTex Pharmaceuticals, Inc.)

Anti-Infectives Would Eradicate Viruses

*As the genetic code began to unravel in the early 1990s, scientists began to explore new options for treating diseases. One possibility involved creating molecules akin to "super-antibodies" that would detect and bind to particular pathogens, flushing them from the body before infection could spread. This new treatment method could significantly reduce healthcare expenditures, could recover worker productivity lost to sick days, and could usher in a new generation of medical treatments designed to eradicate viruses rather than just treat their symptoms. To this end, GelTex Pharmaceuticals, Inc., sought to create custom molecules, called "anti-infectives," that would seek out viruses, bond to them, and render them harmless. The viruses targeted by GelTex were *C. parvum* and human rotavirus, which cause diseases that attack the gastrointestinal tract of individuals with lowered resistance such as children, the elderly, and HIV/AIDS patients.*

GelTex's research could potentially create a pathbreaking technology for curing viruses that would generate substantial economic spillover for employers and the U.S. economy. In 1994, the Advanced Technology Program (ATP) awarded GelTex \$2 million to pursue a three-year effort to develop these anti-infectives. At the end of the ATP-funded project, however, healthcare industry issues had forced GelTex to abandon its research plan. In 2000, Genzyme Corporation acquired GelTex and the two companies continued their drug development efforts. Knowledge that GelTex had gained during the ATP project allowed Genzyme to expedite some drug development functions.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

* *

Research and data for Status Report 94-01-0147 were collected during October - December 2001.

Current Methods Only Treat Symptoms of the Virus

As anyone who has had the flu knows, antibiotics do not work on viruses. Typically, medical professionals can only treat a patient's fever, sore throat, and cough while the virus runs its natural course through the body. Similarly, treatment for the viruses *C. parvum* and human rotavirus, which cause viral gastroenteritis, involved only treating the symptoms of the ailment. Improvements in virus treatments could not be made unless methods of actually killing the viruses themselves were found, which was the focus of GelTek's proposed research.

Killing Viruses Marks a Major Shift for Medical Treatment

GelTex developed a program to research molecular recognition polymers that would act as anti-infectives, binding to the viruses, neutralizing them, and passing them harmlessly through the gastrointestinal tract. Viruses attack by attaching to human cells at specific points, injecting viral ribonucleic acid (RNA) into the human cell where the RNA replicates, forming new viruses, and then moving on to infect other human cells. The proposed anti-infectives would be engineered with an outer "skin" of receptors that would bind to a virus,

occupying the very receptors that bind to and infect human cells. These polymers would be used to neutralize the reproduced viruses, preventing their continued onslaught on the human body. Unable to bond with and infect new cells, the neutralized viruses would either be passed out of the body through waste products or would die inside the body without infecting new cells, thus allowing the patient to recover much faster.

Anti-Infective Technology Could Save Billions of Dollars

In the early 1990s, the healthcare industry projected that the successful treatment of *C. parvum* could save the nation \$135 million annually. Moreover, since rotavirus affects 3.5 million American children annually, better treatment could generate savings of up to \$1 billion in treatment costs and economic losses from parents' lost work time while caring for their children. If this research program proved successful, the next step would be to neutralize other viruses that could result in much greater savings for the U.S. economy. Therefore, ATP awarded GelTex \$2 million to conduct research with the goal of eliminating viruses through the development of its anti-infectives.

Healthcare Industry Concerns Cause Early Termination of Research

During the ATP project, a number of changes in the healthcare industry required that GelTex redirect its research. In the second quarter of 1996, GelTex discovered that a pharmaceutical company was making rapid progress toward introducing a vaccine that would limit the market for an oral rotavirus treatment. At the same time, GelTex was making rapid progress toward developing the *C. parvum* anti-infective. As a result, the company directed its resources away from rotavirus and toward its *C. parvum* program.

Improvements in virus treatments could not be made unless methods of actually killing the viruses themselves were found.

By May 1997, GelTex had identified three lead compounds with which to conduct final *C. parvum*

testing, but again the market shifted in an unfavorable manner for GelTex. The "drug cocktail" of protease inhibitors for HIV/AIDS patients became available in mid-1997. This combination therapy drastically reduced the incidence of *C. parvum* infections among HIV/AIDS patients. With fewer patients catching the virus in the first place, the potential market for a *C. parvum* anti-infective shrank to the \$25 million to \$75 million range. Based on costs to get a drug through the lengthy Food and Drug Administration trials process, this market was too small for investors to pursue. Therefore, with three months remaining on the ATP project, GelTex ceased work on its anti-infective for *C. parvum*.

ATP Award Leads to External Funding

ATP funding lent credibility to GelTex's efforts and aided in attracting additional capital. One year after the initiation of its ATP project, GelTex went public. The company continued to fund infectious disease research after the ATP award ended, more than doubling the staffing level for the program to 14 full-time equivalents. In 2000, GelTex was acquired by Genzyme, and the combined firms used the knowledge that GelTex had gained during the ATP project to continue their drug development efforts.

Conclusion

ATP awarded cost-shared funds to GelTex in order to implement a research plan to develop an "anti-infective" that would eliminate viruses from the human body rather than just treating the symptoms. The healthcare industry and the U.S. economy as a whole stood to benefit from a successful research effort through more effective healthcare and fewer work days lost to illness or time spent caring for sick children. From human rotavirus alone, the economic benefits from successful treatment could reach \$1 billion.

Despite this promising beginning, various changes in the healthcare marketplace led GelTex to abandon its research. Other treatment options for the two viruses tested during the research project entered the marketplace, diminishing the possibility for successful commercialization. After GelTex was acquired by Genzyme in 2000, the knowledge gained from the ATP-funded research assisted the companies' continued drug development efforts.

PROJECT HIGHLIGHTS

Genzyme Corporation (formerly GelTex Pharmaceuticals, Inc.)

Project Title: Anti-Infectives Would Eradicate Viruses
(Molecular Recognition Polymers as Anti-Infectives)

Project: To develop anti-infectives that bind to, and render harmless, *C. parvum* and human rotavirus that attack the human gastrointestinal tract.

Duration: 2/1/1995-1/31/1998

ATP Number: 94-01-0147

Funding (in thousands):

ATP Final Cost	\$2,000	25%
Participant Final Cost	<u>5,860</u>	75%
Total	\$7,860	

Accomplishments: Although changes in the healthcare marketplace ultimately forced GelTex to halt its anti-infective program for rotavirus and *C. parvum*, the ATP project enabled in vivo research on a non-influenza virus for the first time. As a result of the ATP-funded research, GelTex committed significant funds to infectious diseases, more than doubling staffing levels. Further, after returning to rotavirus research in late 1998, GelTex received the following patents:

- "Ionic polymers as anti-infective agents"
(No. 6,034,129: filed June 24, 1996, granted March 7, 2000)
- "Polyvalent polymers for the treatment of rotavirus infection"
(No. 5,891,862: filed September 20, 1996, granted April 6, 1999)
- "Acid-functionalized saccharides as polyvalent anti-infectives"
(No. 5,821,312: filed June 25, 1997, granted October 13, 1998)
- "Ionic polymers as toxin-binding agents"
(No. 6,007, 803: filed September 19, 1997, granted December 28, 1999)
- "Antiviral polymers comprising acid functional groups and hydrophobic Groups"
(No. 6,060,235: filed September 19, 1997, granted May 9, 2000)

- "Polyvalent polymers for the Treatment of Rotavirus Infection"
(No. 6,187,762: filed April 5, 1999, granted February 13, 2001)

- "Anionic polymers as toxin binders and antibacterial agents"
(No. 6,270,755: filed April 3, 2000, granted August 7, 2001)

- "Anionic polymers as toxin binders and antibacterial agents"
(No. 6,290,946: filed May 11, 2000, granted September 18, 2001)

- "Ionic Polymers as Toxin-Binding Agents"
(No. 6,290,947: filed June 19, 2000, granted September 18, 2001)

Commercialization Status: Due to changes in the healthcare industry for *C. parvum* and human rotavirus, GelTex was unable to take its research beyond the laboratory. The knowledge gained from this project has assisted Genzyme (after it acquired GelTex in 2000) in further drug development activities.

Outlook: In late 1998, GelTex renewed its focus on rotavirus research. Some products may well result from the ATP-funded research.

Composite Performance Score: * *

Number of Employees: Seven employees at project start, 14 at project conclusion, 5,000 at Genzyme (which acquired GelTex) as of December 2001.

Company:

Genzyme Corporation
Nine Fourth Avenue
Waltham, MA 02154

Contact: Amy Wilson

Phone: (617) 290-5888

Research and date for Status Report 94-01-0147 were collected during October - December 2001.

Hyseq, Inc.

Prototype DNA Diagnostic Chip to Sequence Entire Genes

Since 1990, scientists have heard the same refrain: DNA-based diagnostics could render "modern medicine" obsolete. Preventive healthcare that would preclude the onset of symptoms from genetic disorders, or would mitigate their effects, could begin at birth. Customized treatments could be used to treat the diseases that do manifest. However, before the new wave of customized, preventative, and post-manifestation treatments could mature and become the norm, physicians needed quick and affordable access to a full map of the patient's genetic code. As of 1995, available DNA diagnostics techniques were too expensive, time-consuming, and inaccurate to effect such a change in modern medicine. At the time, existing diagnostic chips cost thousands of dollars and did not have enough probes to test an entire strand of DNA to fully detect and understand mutations.

In 1994, Hyseq, Inc. was formed to develop the techniques critical for an instrument capable of quickly, inexpensively, and automatically sequencing entire genes using libraries of short DNA probes that hybridize in an overlapping fashion with the target DNA to enable full sequencing. The unique feature of this system is that it is designed to sequence DNA that had not been sequenced before. Other chips were capable of operating only with DNA whose sequence was already known. Hyseq sought venture capitalist funding; however, the funding sources wanted a 70-percent ownership in the company due to the project's extremely high level of risk. In order to help fund this novel initiative for de novo sequencing without giving a significant share of the business to sources of private capital, Hyseq sought cost-shared funding through the Advanced Technology Program (ATP).

In January 1995, ATP awarded Hyseq \$2 million for a two-year project. The ATP project was a scientific success. By late 1997, Hyseq had developed a prototype diagnostic chip that could affordably sequence DNA strands five times as long as any other chip on the market, to greater than 99.99-percent accuracy. While post-project business considerations delayed commercialization, Hyseq's post-project partner, Applied Biosystems, has pledged full support for using Hyseq's chip to develop drugs to treat genetic disorders. In 2001, Hyseq and Applied Biosystems also spun off a majority-owned subsidiary, Callida Genomics, Inc., to pursue the DNA sequencing upon which Hyseq was founded. If Callida is a success, the company could bring new drugs and sequencing methods to market in the next 10 years (the normal drug development timeframe). Even if no drugs result, the ATP-funded project assisted development of a novel method of gene sequencing, resulted in a number of patents, and accelerated screening for new drugs.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

* * *

Research and data for Status Report 94-05-0018 were collected during October - December 2001.

DNA Diagnostics Are Time-Consuming and Expensive

In 1995, traditional DNA (deoxyribonucleic acid) sequencing and diagnostics had two steps, consisting of sample preparation and actual sequencing work.

Interpretation of the DNA sequences was then used in diagnostics. The sample preparation step required producing DNA strands that are either broken-up in a predictable pattern with enzymes or are identified through a reaction that locates specific genetic defects.

Sequencing is done by using a different enzyme reaction that makes a large set of incomplete copies of the DNA. Each of the incomplete DNA copies gets a tag at its end that is used to identify the last base of its DNA strand. This enzyme reaction produces a full set of shortened DNA segments whose length matches each step of the original DNA ladder. The strands are then separated by size using a process called gel electrophoresis. Since the strands naturally carry an electric charge, they move with the current through the gel. Longer strands take more time to worm their way through the gel. It is then possible to "read" the DNA sequence by identifying the tags at the end of the next DNA segment.

Unfortunately, these steps were time-consuming and expensive and required elaborate instrumentation. A new and improved approach was needed to generate the next wave of DNA technologies.

Hyseq's Chip Could Eventually Sequence Entire Genes

The theory behind Hyseq's research efforts was that an entire DNA gene can be sequenced by using a set of short probes composed of every possible DNA sequence. Using the probes in parallel creates a quick and inexpensive process that eliminates many of the obstacles of the conventional process. With those obstacles removed, the separated DNA can then be further examined by sequencing. Small DNA segments are removed from the gel and separately placed onto a test chip covered with probes. The DNA binds to probes on the test chip in a pattern that is then used to read the sequence of the DNA segment. If similar DNA segments with an abnormal or different sequence were tested, it would produce a different binding pattern, leading to the exact identification of the changes in sequence.

Rather than using the industry-standard probe length that requires a million diagnostic probes to analyze a gene, Hyseq scientists developed two sets of 1,000 probes joined together with a ligase enzyme. These sets of probes could detect all possible five nucleotide combinations in parallel. In Hyseq's process, ligase allows the parallel processing of multiple strands of DNA, rather than the one-section-at-a-time serial processing of traditional DNA diagnostic machines.

Hyseq's goal was to develop a prototype sequencing chip that could read and sequence an entire gene. Parallel sequencing was not an industry norm, and Hyseq's chip required hundreds of thousands of chemical and biological processes to generate a successful sequence. This technology was remarkably forward-looking, but presented substantial scientific and business risks.

Scientists Set the Stage for Pathbreaking Technology

A group of veteran scientists from the Department of Energy's Human Genome Project and the sequencing-by-hybridization (SBH) experiments in various national laboratories joined together to submit their ATP proposal offering to form a company, Hyseq, to pursue specific pathbreaking technology. From that proposal, ATP recognized that Hyseq had the potential to create technology that would take the first steps towards altering the course of diagnostic medicine. If successful, Hyseq's plan would enable a new generation of tools for gene sequencing and DNA diagnostic work. Further, this pathbreaking technology could potentially alter the way healthcare is administered, from birth to death, with the implementation of improved preventive care and personalized medication regimens. The proposed HyChip offered the potential to sequence the genetic codes of newborns to encourage a life-long preventive medicine approach that would allow longer and healthier lives, as well as customized medications to combat already-manifested illnesses in the most effective manner.

The theory behind Hyseq's research efforts was that an entire DNA gene can be sequenced by using a set of short probes composed of every possible DNA sequence.

The scientists sought private-sector funding before they formed the company in order to develop SBH processes with private equity. The few venture capitalists who considered funding such a risky endeavor demanded 70 percent of the company in return. Though this was commensurate with the risk of the company, the price was too high to entice these scientists away from their federally funded work with the

Human Genome Project. The \$2 million in cost-shared funds from ATP provided the impetus for them to move into the private sector to form Hyseq. The award, granted to a group of scientists who had not yet formed their company, was held by ATP and disbursed only after Hyseq became a legal entity and achieved an early proof-of-concept by accurately sequencing an industry-standard 64,000-nucleotide string. The ATP funds kept Hyseq's research and development operations going for its first few years.

Further, the award and its follow-on work attracted approximately \$10 million from other funding sources and convinced Hyseq to extend and expand research by 800 percent after the ATP-funded project ended in 1997. According to Mr. Deane Little, Director of Corporate Communications for Hyseq, "The ATP award kept the company going. Without the award, Hyseq never would have done the SBH platform" and never would have developed the HyChip.

This technology was remarkably forward-looking, but presented substantial scientific and business risks.

Spillover was an inevitable component of Hyseq's commercialization plan of forming joint production alliances. Throughout Hyseq's ATP-funded project, executives and scientists spoke at four conferences from 1995 to 1996 and issued a public press release in 1997. They received substantial publicity upon reporting at a biochip conference that they had scored on all one million possible 10-mer¹ probes on sequence samples for HIV and associated test controls. That was more than twice the largest number of probes previously scored by a chip on a sample. By the end of the ATP-funded project, Hyseq had also hired 19 new full-time-equivalent personnel. In addition, the company had filed for seven patents.

Post-Project Bottlenecks Delay Commercialization

Once the HyChip's diagnostic probes were working properly, another problem surfaced. There were no

readers on the market that could process the HyChip's sequences. Hyseq could sequence DNA, but could not read the results or use them to conduct any diagnostic analysis. While large biotechnology companies had readers, they were expensive, incompatible with Hyseq's biochemical processes, and could not interface with Hyseq's database used to detect gene abnormalities. The company did not deal with this problem until after the close of the ATP-funded project. At that time, in 1997, Hyseq needed a partner to develop a HyChip-compatible reader and the diagnostic array technology. Thus, it entered into an agreement with PerkinElmer (now Applied Biosystems). Hyseq executives thought that this agreement would transform their start-up company into an industry powerhouse. The investment community lauded the PerkinElmer-Hyseq partnership and rewarded Hyseq with a successful \$44 million initial public offering during the fourth quarter of 1997.

However, early in the research phase, PerkinElmer redirected its focus away from the technology that had resulted from Hyseq's ATP project. Without the necessary R&D funds, Hyseq could not further develop its array technology. Ironically, a commercial reader compatible with the HyChip became available in late 1998, but Hyseq could not use the reader extensively because of the terms of its agreement with PerkinElmer.

To work around these difficulties, Hyseq used its market capital to fund research efforts to try to extend the HyChip's basic technology to other disciplines. Its efforts included experiments with biological bar codes, nanotechnology applications for diagnostic work, and agents to combat biological warfare. This search for new product lines continued for nearly two years until Applied Biosystems (the newly renamed bioscience section of PerkinElmer) again realized the potential for the HyChip and resumed funding product commercialization. Mr. Little commented that Applied Biosystems' refocused efforts on Hyseq's technology has altered the outlook of the company. In fact, the outlook is "significantly better than it was just a year ago."²

1. mer. This suffix is often used to indicate the number of nucleotides in an oligonucleotide.

2. Interview with Mr. Deane Little, Hyseq's Director of Corporate Communications, summer 2001.

Hyseq Again On the Road to Changing Medicine

For the two years immediately following the conclusion of Hyseq's ATP award, use of the HyChip was limited to internal research. During that time, however, the HyChip achieved some remarkable successes. The chip sequenced the HIV virus correctly on all one million probes, achieved 100-percent accuracy on mitochondrial DNA tests, and sequenced 500 percent more bases than was possible with a traditional DNA diagnostic chip. The potential arising from these post-ATP tasks is far-reaching.

ATP recognized that Hyseq had the potential to create technology that would take the first steps towards altering the course of diagnostic medicine.

The HyChip enables researchers to generate sequences of, and develop other large data sets from, genes within a given species. Taken over a large population, this data can then be used to correlate genotype to phenotype (genetic code to physical traits). Once the database of genotype/phenotype correlations is completed for humans, the knowledge generated could usher in a new age of preventive healthcare, beginning with an individual genetic sequence performed immediately after birth. Working with Applied Biosystems, the HyChip can be used as part of the process to develop drugs both as preventative measures to keep genetic defects from developing into full-blown illnesses and also to treat those illnesses on a customized basis once they do manifest. Drug development is a long process that spans 8 to 10 years before a successful drug can reach the market. It is anticipated that in the next 10 to 15 years, the HyChip will help bring new drugs to market. As of 2001, Hyseq and Applied Biosystems had spun off a subsidiary, Callida Genomics, to pursue additional advances in DNA sequencing technology.

Conclusion

ATP awarded Hyseq \$2 million to help develop a DNA diagnostic tool for sequencing entire genes at once. Hyseq succeeded in developing a prototype sequencing

chip, called the HyChip, during the ATP project. Shortly after the ATP project ended, however, an open-ended partnership agreement between Hyseq and Applied Biosystems resulted in a two-year delay of HyChip commercialization efforts. Fortunately, post-ATP project research and development and commercialization efforts are now going forward under a separate company, Callida Genomics, that was spun off from Hyseq and corporate partner Applied Biosystems. With Callida, the Hyseq ATP-funded technology is once again being utilized to promote advances in DNA diagnostics.

PROJECT HIGHLIGHTS

Hyseq, Inc.

Project Title: Prototype DNA Diagnostic Chip To Sequence Entire Genes (Sequencing By Hybridization Format 3 Megabase Diagnostics Instrumentation)

Project: To develop an instrument capable of quickly, inexpensively, and automatically sequencing entire genes using libraries of short DNA probes that hybridize in overlapping fashion with the target DNA to enable sequencing of an entire gene at once.

Duration: 1/1/1995-12/31/1997

ATP Number: 94-05-0018

Funding (in thousands):

ATP Final Cost	\$2,000	57%
Participant Final Cost	<u>1,498</u>	43%
Total	\$3,498	

Accomplishments: Using ATP funds, Hyseq developed a prototype called the HyChip that could sequence entire genes at one time, eliminating the need for many costly and time-consuming preparation steps.

The following are among HyChip's successes:

- Scored correctly on all one million probes with HIV sequence samples
- Scored 100-percent accuracy on mitochondrial DNA tests
- Sequenced 500 percent more bases with one chip than with traditional diagnostic chips

The accomplishments of this ATP-funded project led to the following patents:

- "Method of sequencing of genomes by hybridization of oligonucleotide probes"
(No. 5,667,972: filed June 5, 1995, granted September 16, 1997)
- "Method of sequencing by hybridization of oligonucleotide probes"
(No. 5,695,940: filed June 5, 1995, granted December 9, 1997)

- "Methods and compositions for detection or quantification of nucleic acid species"
(No. 6,309,824: filed January 16, 1997, granted October 30, 2001)
- "Methods for sequencing repetitive sequences and for determining the order of sequence subfragments"
(No. 6,297,006: filed October 2, 2001, granted March 4, 1997)
- "Method of sequencing of genomes by hybridization of oligonucleotide probes"
(No. 5,667,972: filed July 29, 1997, granted January 25, 2000)
- "Methods and compositions for detection or quantification of nucleic acid species"
(No. 6,383,742: filed August 15, 1997, granted May 7, 2002)
- "Reagent transfer device"
(No. 5,882,930: filed November 10, 1997, granted March 16, 1999)
- "Reagent transfer device"
(No. 6,255,119: filed September 25, 1998, granted July 3, 2001)

Knowledge spillover resulted through Hyseq's partnership with a university to conduct research and development, its joint venture with PerkinElmer, and presentations it made at biochip conferences.

Commercialization Status: Before the ATP project, Hyseq had no commercializable products, just a research idea. At project closeout, Hyseq had developed a prototype DNA diagnostic chip, called the HyChip, that could sequence an entire gene at once. Hyseq also had entered into an exclusive agreement with PerkinElmer, a major pharmaceutical company, to develop and commercialize the HyChip. Due to internal business decisions made several months after the end of Hyseq's ATP project, PerkinElmer changed course and did not fund the development of the HyChip. By 2000, PerkinElmer committed itself as a company to bioscience, renaming its bioscience division Applied Biosystems. As part of the bioscience focus, Applied Biosystems began using Hyseq's technology in its drug development efforts. In 2001, Applied Biosystems and Hyseq spun off a subsidiary, Callida Genomics. Callida, which inherited all the intellectual property related to Hyseq's ATP-funded project, will pursue the DNA sequencing technology upon which Hyseq was founded.

Outlook: Applied Biosystems has once again pledged full support for using the HyChip to develop drugs to treat genetic disorders. Once a compound starts through the Food and Drug Administration approval process for new medications, final approval is still at least eight years away. Identifying a compound to send through the approval process, however, is time-consuming. Therefore, if Hyseq and Applied Biosystems' support remains constant for their spinoff Callida, it is possible that Callida technology will impact the development of new therapeutics. Until that time, however, the outlook is uncertain.

Composite Performance Score: * * *

Number of Employees: 12 employees at project start, 156 as of December 2001.

Focus Program: Tools for DNA Diagnostics, 1994

Company:

Hyseq, Inc.
670 Almanor Avenue
Sunnyvale, CA 94086-3513

Contact: Deane Little

Phone: (408) 524-8100

Research and data for Status Report 94-05-0018 were collected during October - December 2001.

Nanogen, Inc.

Microchip To Speed DNA Analysis Process

Whether or not a patient will respond well to a particular drug is dependent on his or her genetic composition. In 1995, however, there were significant barriers to performing diagnostic tests using DNA samples. Machines capable of DNA diagnostic work were large, expensive, cumbersome, and in short supply. Moreover, they required lengthy sample preparation work before the diagnostic process could even begin. This dearth of available, cost-effective processes often prevented the use of DNA sequencing in diagnostic work. Given the promise of DNA-based diagnostics, industry leaders indicated that the question was not whether diagnostic work would adopt a DNA-based approach, but when the adoption would begin. The Advanced Technology Program (ATP) hoped to accelerate that shift through a focused competition entitled "Tools for DNA Diagnostics." Nanogen responded by submitting a proposal to develop a microlaboratory (a diagnostic lab on a microchip) for DNA diagnostics that would reduce the previously cumbersome sample preparation steps for DNA to a single, rapid process carried out on a single microchip.

In August 1995, ATP awarded Nanogen funds to pursue the development of its DNA diagnostic sample preparation system. Nanogen successfully reduced sample preparation time significantly and created a microlaboratory, which represented the first step towards making DNA diagnostics affordable and effective. Through the assistance of a separate ATP grant, Nanogen narrowed the microlaboratory's focus and reduced the size and cost of the machine in order to commercialize a product. As of February 2003, Nanogen reported product revenues of \$3.4 million, driven by two staple products, the NanoChip® workstation and the NanoChip® microarray.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

* *

Research and data for Status Report 95-08-0009 were collected during June 2001 - December 2002, and February 2003.

Potential To Harness the Human Genome Project for Improved Healthcare

Since 1990, the National Institutes of Health and the Department of Energy have been funding the Human Genome Project to sequence and map out the thousands of individual genes strung along the 46 chromosomes in human cells. The human genome map was expected to provide a multitude of insights into poorly understood diseases and biological phenomena, as well as new treatments for genetically based ailments. DNA diagnostics would link the information learned from the gene map to personalized medical treatments for each patient.

As the Human Genome Project opened up new horizons and possibilities for the diagnosis and treatment of diseases, scientists needed fast, reliable diagnostic tools that were inexpensive enough to fit into healthcare budgets. To that end, ATP took a lead role in funding the technology's development. In 1992, the in vitro portion of DNA diagnostics (tests performed outside the patient's body) had a market size of \$58 million. The entire diagnostics market was \$5 billion. ATP funding helped in vitro DNA diagnostics to become a more mature, competitive industry with many new products. In fact, by 1997, the market size for diagnostics was \$20 billion, with in vitro DNA diagnostics accounting for more than \$500 million of that amount.

While the potential for growth in the DNA diagnostics market was predictable, it was not clear exactly what apparatuses would come to market and fuel that growth. Nanogen's plan to use electric current to attract DNA to the capture probes had the potential to speed up the process of sample preparation, making DNA diagnostic work faster and less expensive.

In 1995, there were significant barriers to performing diagnostic tests using DNA samples.

Nanogen's proposal to ATP represented a critical step in developing an enabling technology to use the knowledge gained from the Human Genome Project to generate better, cheaper, and more accurate DNA diagnostic work. In 1995, ATP awarded Nanogen two years of cost-shared funds totaling \$2 million to give the company a chance to advance its promising technology plan.

Existing Diagnostic Methods Are Slow and Costly

In 1995, DNA diagnostic work required significant sample preparation before the process of analyzing the code and diagnosing ailments could begin. The preparation process typically involved cutting the strand with particular enzymes and running the strands through a gel using electric current. Since DNA itself carries a negative charge, it follows the current and works its way from the negative end of the gel to the positive end of the gel. Longer strands take longer to work their way through the gel molecules; therefore, the DNA separates by size into a predictable pattern. The particular portion of interest within particular strands then had to be separated and prepared for analysis. The strands with the appropriate genes could then be compared against other like strands to test for defects.

In 1995, there were products on the market that could run 96 gels in parallel, separate the DNA in 90 minutes, and use a computer to read the results directly from the gels. Older technology still on the market took up to six hours for the DNA to separate in the gel. Each of these technologies, however, required significant preparation work to further separate and amplify particular DNA segments.

Microchip and Biological Advances Could Slash Sample Preparation Time

Nanogen proposed to develop an integrated system of microelectronic components that rely on electronically controlled properties of cells and molecules to achieve separation, selectivity, amplification, and identification of medically relevant DNA sequences. In the proposed Nanogen process, a sample (usually a drop of blood) would be placed onto the chip. A cell selector site would then exploit the different binding affinities of different cell types under different electric conditions to select the appropriate cells for analysis.

The next site on the chip would rely on the same concept of binding affinity to separate and extract relevant DNA from the other components of the selected cells. Yet another site would pass the DNA over an array of probes designed to locate, bond with, and identify genetic material associated with defects or infectious diseases. Finally, each selected sample fragment would be analyzed in detail for specific genetic mutations or patterns. If any specific defects were found, a dye would bind to that location. From the pattern of colors on the chip, diagnostic scientists could potentially infer the sample DNA's sequence and diagnose conditions or prescribe treatment from that sequence.

A computer capable of reading and understanding the pattern of dyes on diagnostic chips would be required for Nanogen's innovation to become commercially viable. Nanogen proposed to create a microelectronic DNA diagnostic system contained on one microchip. If successful, the sample preparation step in DNA diagnostics would be eliminated, making the process significantly less time-consuming.

Nanogen Explores Potential of Enabling Technology

Nanogen's proposal to create a diagnostic lab on a microchip held the potential to develop the enabling technology of rapid sample preparation for DNA-based diagnostics. While other technological advances would be necessary to analyze the results of sample preparation in order for affordable DNA-based diagnostics to come to market, Nanogen's research

plan would enable those other technological advances by setting a common standard for sample preparation. If the project succeeded, developers of sample analysis machines would be able to start product development from a defined standard for samples and work toward a universal reader for DNA diagnostics. This would save time and money throughout the entire DNA diagnostics industry.

Nanogen proposed to create a microelectronic DNA diagnostic system contained on one microchip.

ATP funds were necessary to support this research because Nanogen proposed a high-risk use of electricity, chemistry, and microfluidics that might not succeed. As a start-up company, Nanogen could not afford to fund the development of an integrated system for DNA diagnostic machines; instead, it focused most of its spending on supporting its existing products. Even if it did succeed, Nanogen could not reap all the economic benefits of the standard they would set for the DNA diagnostic industry. A successful project could enable spillover effects across many economic sectors by combining the knowledge from the Human Genome Project with innovations in microfluidics and computing power to create an entirely new industry.

Nanogen Solves DNA Diagnostic System Technical Issues

Because Nanogen planned to create an entirely new diagnostic industry, the company realized early that it had to build a new type of DNA diagnostic machine from the ground up. With ATP funding, Nanogen sought to merge a microchip, electric current, waveguides, and low-power lasers into an integrated microelectronic DNA diagnostic system. Nanogen had to overcome three major technical problems in order to achieve success.

The first, overarching technical challenge for Nanogen scientists was to create a microchip that could reduce or eliminate sample preparation times. Taken separately, the components of this chip were not remarkable. Integrating knowledge and applications from a diverse array of industries and enabling them to

operate on a high throughput chip was a remarkably risky technical endeavor. By circumventing or solving technical obstacles as they arose throughout the course of the ATP-funded project, Nanogen achieved its ultimate goal of reducing sample preparation times from hours to minutes. Moreover, much of the sample preparation work was automated, freeing researchers to do other research rather than work on time-consuming sample preparation.

The second technical challenge was to find a way to fit a waveguide small enough to focus a low-power laser onto the DNA diagnostic chip so that it could accurately read the data, but not destroy the sample. Electric current had not been used to separate DNA prior to the Nanogen project. Lasers and waveguides available at the time of the ATP-funded project would have sent too much current through the sample, essentially electrocuting the biological sample. To solve this problem, Nanogen experimented with different types of waveguides and laser frequencies used in other industries until scientists found a combination that functioned properly and did not destroy the sample. The resulting waveguide was a new application for existing technology.

The third technical challenge, addressed toward the end of the ATP-funded project, involved expanding the screening ability of Nanogen's microchip. The early prototype diagnostic chip was incapable of screening DNA segments with more than just a couple of genetic defects. Screening for a large number of defects is critically important for a DNA diagnostic system because genetic abnormalities often result from four or five defects on a gene, rather than just one. Each defect must be located, replicated and amplified with a polymerase chain reaction, marked with dye, and then run through a diagnostic machine. The early diagnostic chip's prototype camera became fooled by a large number of dye-marked defects and recorded interference rather than an accurate picture of genetic abnormalities.

In the final year of the ATP-funded project, Nanogen succeeded in implanting better capture probes using improved chemistry and electronic circuitry for active hybridization. The result was that Nanogen scientists "multiplexed" four areas on the chip and partitioned it in a way that prevented interference.

Nanogen reduced the time needed for the entire process, from preparation through amplification to analysis, from hours to minutes and eliminated the extensive sample preparation work.

Conclusion

In 1995, Nanogen scientists devised a research plan to create the generic technology for a DNA diagnostic microlaboratory and received funding from the ATP focused program "Tools for DNA Diagnostics." The project's objective was to combine previously cumbersome sample preparation steps with an analysis chip that could substantially reduce the time and expense of analyzing DNA. Nanogen succeeded in combining the sample preparation and analysis steps into one process that took minutes instead of hours and freed up scientists to focus on other research instead of preparation work. Nanogen developed a DNA microlaboratory through additional research after the close of this ATP-funded research. As of February 2003, the company is generating annual product revenues of \$3.4 million from sales of its NanoChip® array and a workstation.

PROJECT HIGHLIGHTS

Nanogen, Inc.

Project Title: Microchip To Speed DNA Analysis Process
(An Integrated Microelectronic DNA Diagnostic System)

Project: To speed the entry of cost-effective DNA analysis into the clinical diagnostic laboratory through the development of an integrated system to carry out all necessary sample preparation and analytical procedures in a linked series of microfabricated sites that sum into a microlaboratory on a single chip.

Duration: 8/1/1995-12/31/1997

ATP Number: 95-08-0009

Funding (in thousands):

ATP Final Cost	\$2,000	57%
Participant Final Cost	<u>1,500</u>	43%
Total	\$3,500	

Accomplishments: Nanogen developed a microchip-sized DNA preparation system that uses electric current to actively hybridize DNA, allowing for faster and less expensive DNA diagnostic work. The highlights of this technology, and its spillover effects, include:

- Development of a small DNA diagnostic chip that quickly and effectively prepares DNA and conducts diagnostic analysis in minutes instead of hours.
- Incorporation of waveguides and low-powered laser light into a microchip to create an active hybridization process that allows DNA to be prepared for analysis significantly faster than before. By automating the sample preparation process, Nanogen freed researchers to conduct research rather than prepare samples.
- Publication of an article written by Nanogen scientists in the April 2000 issue of Nucleic Acids Research entitled, "Rapid, High Fidelity Analysis of Simple Sequence Repeats on an Electronically Active DNA Microchip."

Nanogen applied for three patents (two were granted), with the accompanying disclosure of technical innovations as part of the patent application process. The patents Nanogen received from this project are:

- "Apparatus and methods for active biological sample preparation"
(No. 6,129,828: filed September 6, 1996, granted October 10, 2000)
- "Channel-less separation of bioparticles on a bioelectronic chip by dielectrophoresis"
(No. 6,071,394: filed January 30, 1998, granted June 6, 2000)

Commercialization Status: Nanogen is continuing research into developing the necessary computer system, optical-imaging capabilities, and databases of genetic sequences that would enable commercialization of the portable genetic analysis system. As of February 2003, Nanogen was generating \$3.4 million in product revenues per year from products such as the NanoChip® array and the workstation.

Outlook: The market for DNA diagnostic machines is expected to increase substantially if other technologies become available to harness the power of Nanogen's innovation. Until those technologies appear, however, the outlook is uncertain.

Composite Performance Score: * *

Number of Employees: 48 employees at project start, 160 as of December 2002

Focused Program: Tools for DNA Diagnostics, 1995

Company:

Nanogen, Inc.
10398 Pacific Center Court
San Diego, CA 92121-4385

Contact: Dr. Marc Madou

Phone: (877) 626-6436

Nanogen, Inc.

Briefcase-Sized System for Accurate, Cost-Effective DNA Diagnostics

By 1997, the various tools that used DNA as the basis for diagnostic work were progressing toward the commercialization stage. Several prototypes existed, but none had the crucial combination of small sample preparation cycle times and miniaturized components. Existing processes were labor intensive and required a significant number of additional steps to complete a DNA-based diagnostic workup. Consequently, DNA diagnostic tests cost between \$3,000 and \$20,000 in 1997 because of the lack of affordable analysis technology and processes. Using cost-shared funds awarded by the Advanced Technology Program (ATP) in May 1997, Nanogen, Inc., sought to develop a briefcase-sized analysis system that could accept standardized, prepared DNA samples and generate accurate diagnostic results at a commercially viable cost of \$100 per test. Nanogen's plan was to expand its core DNA analysis microchip technology to the point where the chip could integrate with an entirely portable analysis unit and provide results for any desired DNA sequence. In late 1999, when the ATP-funded project ended, Nanogen had successfully developed a prototype DNA diagnostic machine that reduced sample preparation time from 40 minutes to 10 minutes and reduced the cost to just over \$100 per test.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

* * *

Research and data for Status Report 96-01-0172 were collected during June 2001 - December 2002.

Diagnostic Methods Are Too Slow and Too Costly

In late 1996, there were products on the market that could analyze 96 gels in parallel, separate the DNA in 90 minutes, and use a computer to read the results directly from the gels. Older technology still on the market took up to six hours for the DNA to separate in the gel. Though the newer technology offered the improvement that was required for diagnostic work, the cost was still too high for widespread use, with DNA diagnostic tests ranging from \$3,000 to \$20,000 each.

Even with the improvements in DNA diagnostics that had been achieved during the early 1990s, several barriers were still evident in late 1996. First and foremost, no DNA reader existed that could analyze large samples prepared on microchips, detect abnormalities, cross-reference the abnormalities with a database of appropriate DNA sequences, and suggest proper diagnoses. Second, large samples could not be sequenced without time-intensive preparation work and analysis on multiple chips. Third, materials and

manpower costs remained too high for chip-based DNA diagnostics to achieve widespread acceptance within the healthcare industry.

These were significant problems since genetic disorders are sometimes caused by multiple genetic abnormalities. In order to ensure an accurate diagnosis, scientists need to sequence large amounts of DNA. Before this ATP-funded project, analyzing large amounts of DNA could not be done on one chip. As a result, DNA diagnostic technology was not as accurate or cost-effective as was required for commercial viability.

Proposed Technology Could Significantly Reduce Costs

Nanogen's proposal to ATP focused on overcoming the technical barriers that stood in the way of a briefcase-sized, portable genetic analysis system that could rapidly, accurately, and inexpensively analyze a genetic sequence. The primary application for the system would

be detecting pathogens or defects for medical diagnostics and epidemiology. The system would replace labor-intensive analysis steps that increased analysis costs significantly more than was necessary. The proposed analysis system would be able to take a standardized sample of any size, fragment and separate the appropriate nucleic acids, and determine where the abnormalities were in the specific genetic code.

The key innovation, in addition to dramatic improvements in sample preparation and analysis, was the creation of a multichip detection and control module to analyze the assay results. The proposed ATP project would significantly extend Nanogen's core abilities to prepare samples and conduct the sequencing in order to handle larger genetic samples quickly, accurately, and inexpensively.

Nanogen's proposal to ATP focused on a briefcase-sized, portable genetic analysis system.

By bringing the cost of DNA-based diagnostics down to a commercially viable target of \$100 per test, a successful Nanogen project would create economic benefits for healthcare providers, employers, and patients, as well as positively impact worker productivity and general public health. If DNA diagnostics became less expensive, healthcare providers could administer the more accurate, more efficient tests and could begin preventative treatment immediately. Employers could keep their health costs down and, by providing preventive medical care benefits, could avoid longer term, more serious illnesses in employees. Overall productivity could increase because a healthier population takes fewer sick days, and a general improvement in public health could prolong life.

Technical Risks Forestall Funding

Nanogen's proposed approach involved completely overhauling its central systems and chips in order to integrate them into a new set of hardware and software. The company would have to divert significant resources to merge so many disparate systems, which would lead to a negative impact on other shorter term efforts. As an

early- to mid-stage company, Nanogen could not devote the necessary resources to the high-risk integrated technologies needed for a next-generation system. The risk, coupled with the lack of private-sector funding, led Nanogen to submit a proposal to ATP. In 1997, ATP awarded the company \$2 million to develop a portable genetic analysis system.

Nanogen Solves Technical Issues with the DNA Diagnostic System

In order to miniaturize the technology and create a briefcase-sized DNA diagnostics system, Nanogen had to improve the sample preparation process and adjust its core chip technology to separate and analyze substantially more DNA. Across the life of the ATP-funded project, Nanogen scientists and engineers worked hard to address these technical shortcomings. By the end of the project, Nanogen had shortened the sample preparation cycle from 40 minutes to 10 minutes by creating a new amplification and preparation system. The company also improved its diagnostic chip so that the chip could handle more prepared samples than it could before the start of the project. Finally, Nanogen adjusted the electric current running through its chip to optimize the process and complete the briefcase-sized DNA diagnostic system.

DNA Diagnostic Product Is Commercialized

Through the ATP-funded project, Nanogen developed a working prototype that accurately sequenced DNA samples. Knowledge gained from that prototype led to the commercialization of the NanoChip™, a chip reader, and a chip loader. The NanoChip™ itself is only 0.7 square centimeters, but up to 109 cell fragments per site on all 99 test-sites can be loaded onto the chip. Even with such an intense load, the chip and the reader can accurately separate, hybridize, and sequence up to 400 base pairs from up to 99 trillion cell fragments. After equipment purchase, the total cost per test is just over \$100—significantly less than the \$3,000 to \$20,000 per test that was charged before this project began.

The entire NanoChip™ Molecular Biology Workstation contains three separate subsystems: (1) a freestanding microchip loader with a fluid-handling subsystem to perform electric addressing of blank microchips in

microchips in preparation for specific types of samples; (2) a highly sensitive, laser-based fluorescence scanner; and (3) computer hardware and software with a graphical user menu. The first and third systems contain significant amounts of knowledge gained during this research project.

The resulting technology reduced costs from a high of \$20,000 to about \$100 per test and reduced the time needed for sample analysis by 75 percent.

Nanogen scientists published several papers and gave a number of presentations on components within the workstation. The company also disclosed information specific to its technical advances in seven patent applications stemming from this ATP-funded project.

Conclusion

Nanogen set out to create a DNA diagnostic system that could prepare, process, and analyze samples more quickly, more accurately, and less expensively than machines on the market in 1997. ATP cost-shared funding enabled Nanogen to successfully achieve this goal. The resulting technology reduced costs from a high of \$20,000 to about \$100 per test and reduced the time needed for sample analysis by 75 percent.

PROJECT HIGHLIGHTS

Nanogen, Inc.

Project Title: Briefcase-Sized System for Accurate, Cost-Effective DNA Diagnostics (A Portable Genetic Analysis System)

Project: To develop a portable genetic analysis system that can rapidly and accurately profile a genetic sequence for applications including forensic analysis, battlefield casualty identification, trauma victim identification, diagnostics, and environmental and health monitoring.

Duration: 5/1/1997-9/30/1999

ATP Number: 96-01-0172

Funding (in thousands):

ATP Final Cost	\$2,000	51%
Participant Final Cost	<u>1,935</u>	49%
Total	\$3,935	

Accomplishments: This highly successful program enabled a biotech startup company to develop a microchip-sized DNA diagnostic system and a briefcase-sized workstation for inexpensive analysis. The company's technology reduced the total cost of tests from a high of \$20,000 per test to just over \$100. The cost savings resulted from the decreased size, energy, process, and manpower requirements facilitated by the system's automated processes. Nanogen applied for seven patents for this technology; the applications were still pending at the time of the Status Report research.

Commercialization Status: The NanoChip™ Molecular Biology Workstation is the first product line in what Nanogen hopes will be a long series of DNA diagnostic tools. Current capabilities include detecting abnormalities in gene sequences. Future diagnostic applications include entries into the oncology, infectious diseases, and genetic testing markets.

Outlook: As the benefits of the Human Genome Project become more widespread, the market for DNA diagnostic machines will increase substantially. Moreover, as Nanogen endeavors to develop affordable and smaller diagnostic machines for increasingly diverse illnesses and applications, the market will expand. Nanogen created the first stand-alone diagnostic system that could perform inexpensive tests of large DNA samples. Therefore, the outlook for Nanogen, and for the DNA diagnostic industry, is good.

Composite Performance Score: * * *

Number of Employees: 48 employees at project start, 160 as of December 2002.

Company:

Nanogen, Inc.
10398 Pacific Center Court
San Diego, CA 92121-4385

Contact: Dr. Marc Madou

Phone: (877) 626-6436

Research and data for Status Report 96-01-0172 were collected during June 2001-December 2002.

Third Wave Technologies, Inc.

Cleavase Technology Reduces Cost and Shortens Time of Genetic Analysis

By 1994, scientists were generating vast amounts of genetic sequence data, but they lacked a low-cost, user-friendly system to analyze that data and translate it into a format useful for research and diagnostic work. The analysis systems that were available for determining genetic differences between individuals required expensive and extended procedures under highly controlled conditions. Prior to 1994, scientists from Third Wave Technologies, Inc., a company of three researchers, had discovered a promising new enzyme technology that had the potential to facilitate the more rapid, inexpensive, and simplified analysis of individual genetic variabilities. Third Wave's Cleavase technology held the potential to reduce the cost of genetic analysis by up to 90 percent and shorten the analysis time from days to hours.

Private capital sources viewed the technology, and the company, as too high risk for investment. However, the proposed innovation, cost savings, and potential for creating a new tool for genetic analysis met the criteria to receive cost-shared funds for a two-year research project as part of the Advanced Technology Program's (ATP) "Tools for DNA Diagnostics" focused program. By the end of the ATP-funded project, three generic genetic analysis tools had been created. The company had also presented its findings at a dozen conferences and had devoted substantial additional funds to continue the research and development of its Cleavase-based products. In 2001, Third Wave earned more than \$34 million in revenues and conducted a successful initial public offering.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

* * * *

Research and data for Status Report 94-05-0012 were collected during December 2001 and December 2002 - January 2003.

Scientists Lack Methods To Analyze Genetic Data

The genetic revolution was well underway in 1994. Researchers were looking for ways to identify new genes and genetic mismatches between those genes. Genetic mismatches are a basis of genetic diversity and carry significant information with regard to the cause of disease and normal development. Mismatches are common, and most of the differences between individuals are differences in single nucleotide polymorphisms (more commonly known as SNPs). Researchers in the public and private sectors spent considerable effort determining how to analyze SNPs and other types of genetic mismatches in order to accelerate the rate of research and discovery within the diagnostic and therapeutics fields.

In 1994, no commercially viable method existed for analyzing genetic mismatches. In order to determine if these genetic mismatches were present, scientists had to perform time-consuming and expensive analyses, which involved either resequencing the DNA using polymerase chain reaction (PCR, which is a process by which a specific region of DNA is amplified by DNA synthesis enzymes working from the two "primer" ends banding the area to be amplified and working inwards) or using a process known as restriction fragment length polymorphism (RFLP) determination. RFLP is a technique in which organisms may be differentiated by an analysis of the patterns derived from cleaving their DNA. In order to perform PCR, scientists still needed to perform extensive testing to detect and separate the desired DNA strands. RFLP analysis also required

many time-consuming and complicated steps. As the Human Genome Project advanced and continued to generate volumes of genetic data, scientists needed a faster process for analyzing gene sequences.

Cleavase Technology Could Potentially Bridge the Gap

Nucleic acid sequence data for genes were accumulating through the Human Genome Project and other sources. Scientists needed fast, cost-effective, and easy-to-use tests for the detection of mutations. In 1993, Third Wave developed a system that included novel methods and enzymes for the targeted detection and cleavage of DNA and RNA. The basis for Third Wave's proposed toolbox derived from a set of newly discovered bacterial enzymes that fell under the company's trade name Cleavase, but that had yet to be fully developed. The key principle behind the Cleavase enzymes is that they can easily be molecularly tuned to cleave any predetermined sequence of nucleic acids. Successful exploitation of the unique features of Third Wave's Cleavase enzymes could potentially lead to a major breakthrough in nucleic acid detection capability, but further research was required.

In 1994, no commercially viable method existed for analyzing genetic mismatches.

Third Wave scientists believed that a fully developed Cleavase-based DNA analysis system would provide a fast and inexpensive means of obtaining biological and human genetic information. Research and diagnostic answers could be available in a few minutes instead of hours or days. Moreover, the cost of Cleavase-based diagnostics was expected to be 10 to 20 percent of the cost of the systems on the market in 1994. As a result, Cleavase-based products could offer effective new ways to facilitate therapeutic decisions based on individual genetics, could help accelerate the rate of research and discovery in general, and could help reduce U.S. healthcare costs by making widespread, preventive diagnostic screening less costly.

ATP Funding Necessary To Explore Cleavase's Properties

Third Wave was founded in October 1992 to develop new methods of analyzing genetic material. Less than one year later, the company created its basic Cleavase technology, but as an early-stage company, Third Wave did not have ready access to capital that could be used to fully research Cleavase's capabilities. In its proposal to ATP, Third Wave's CEO, Dr. Lance Fors, commented that without ATP funding, Third Wave would have to abandon development of the potentially powerful generic Cleavase technology in favor of focusing on developing one or two DNA diagnostic kits for specific applications. With ATP support, however, Third Wave would be in a position to increase the rate of research by at least a factor of four. At the time of Third Wave's 1994 application, sources of private capital viewed the company's technology as too high risk for private funding. Because Third Wave was the only company pursuing this technology, it would not advance without ATP support.

Enormous Potential Market Existed for Cleavase-Based Products

The Cleavase technology, if developed successfully, would allow any target DNA or RNA sequence to be detected and cleaved at any desired position in a fast, automated, user-friendly, low-cost fashion that would make large-scale screening possible for the first time.

Research and diagnostic answers could be available in a few minutes instead of hours or days.

In 1994, there was strong and immediate demand for this technology in the research marketplace from more than 400,000 life science researchers in 55,000 research laboratories worldwide. Cleavase-based diagnostic products would also find strong demand in hospital testing laboratories, clinical laboratories, and doctors' offices. Applications included low-cost, kit-based diagnostic products for DNA typing; military and

criminal identification; forensic science; paternity testing; food processing test applications like monitoring for E.coli in beef, agriculture, and animal husbandry; and changes in DNA and RNA sequences or RNA expression levels in response to environmental changes.

Cleavase Technology Resulted in Two Specific Applications

Third Wave used part of the ATP funds to create a process that was at least as effective as RFLP. The new process, known as Cleavase Fragment Length Polymorphism (CFLP), generated a distinct bar code for every unique DNA sequence. Thus, genetic mismatches could be detected by comparing a sample to a normal coding pattern. The method was expected to have a broad range of applications for research, the diagnosis and treatment of infections and hereditary diseases, and the acceleration of drug development. For example, CFLP could distinguish between different strands of the bacterium that causes tuberculosis. This meant that doctors would be able to treat specific patients with tailored drug treatments that would minimize drug-resistance problems.

The rest of the ATP funds were used to develop a preliminary assay that would later become the "gold standard" for non-polymerase chain reaction-based (non-PCR-based) SNP detection due to its speed, accuracy, and relative ease of use. Using this assay, Third Wave developed a rapid non-PCR-based SNP detection system that works by invading the normal DNA duplex with a third piece of single-stranded DNA. Hence, the system became known as the Invader system. In the Invader process, two short DNA probes hybridize to the target to form the structure recognized by the Cleavase enzyme. When the proper structure is formed, the enzyme then cuts one of the probes to produce a target-specific fluorescent signal. Each target generates thousands of signals per hour, yielding millions of detectable signals per target. In short, it brings detection levels in line with those of PCR without requiring the post-amplification tests to separate the genetic material that PCR requires.

After this ATP-funded project was complete, Third Wave sought and received additional research money from public and private sources to further develop the CFLP and Invader technology. In 1997, Third Wave

received another ATP award to build genetic detection tools for healthcare applications. This research ultimately led to a robust product line. The enhanced Invader assay began to generate revenues in 2000. That year, Third Wave earned \$11.4 million in revenues. In 2001, revenues increased to \$34.1 million, and with cost of goods sold at \$32.9 million that year, Third Wave finally became profitable. On the strength of these revenues, the company held an initial public offering (IPO) in 2001, which raised \$82.5 million for Third Wave. With the economic downturn, however, Third Wave found it difficult to maintain profitability. Nevertheless, the Invader assay continues to be the gold standard for anyone engaged in non-PCR analysis.

Third Wave Shares Project Knowledge

Third Wave shared a significant amount of the knowledge it gained through the ATP-funded project. The company has published more than 20 papers and has presented more than 30 posters. Third Wave representatives also made presentations at 12 conferences during 1995 and 1996 to showcase the information learned from the ATP-funded research. In addition, various newspapers, magazines, and trade press published six articles about Third Wave's ATP-funded technology in 1995 and 1996 and numerous additional articles since then. Third Wave has 10 issued U.S. patents flowing from technology developed during the project. Moreover, the successful project substantially improved Third Wave's credibility, which assisted company executives in obtaining additional capital and in conducting the 2001 IPO.

Conclusion

In 1994, scientists sought to shorten the time required to analyze genetic data. Third Wave applied to ATP for award money to fund their research into this new method of genetic analysis. In their research plan, they proposed to step outside traditional thinking and develop an entirely new method of analyzing segments of genetic material. By the close of the ATP-funded project, Third Wave had created a new method of analyzing DNA and had disseminated that knowledge through publications and patent applications. Third Wave conducted an initial public offering and continues to produce DNA analysis machinery.

PROJECT HIGHLIGHTS

Third Wave Technologies, Inc.

Project Title: Cleavase Technology Reduces Cost and Shortens Time of Genetic Analysis (Development of a Generic Technology for the Targeted Detection and Cleavage of DNA and RNA)

Project: To develop simple-to-use, low-cost diagnostic tools that rapidly detect specific DNA and RNA sequences for broad-based medical diagnosis and for tracking treatments.

Duration: 1/1/1995-12/31/1996

ATP Number: 94-05-0012

Funding (in thousands):

ATP Final Cost	\$1,998	72%
Participant Final Cost	<u>771</u>	28%
Total	\$2,769	

Accomplishments: During this research project, Third Wave grew from a company of 3 researchers to a staff of 195. The ATP-funded program brought Cleavase technology to the point where it could be developed into prototypes and specialized into different products.

Technology developed during this ATP-funded project also led Third Wave to apply for 11 patents (of which 10 have been issued) for its Cleavase Fragment Length Polymorphism (CFLP) and Invader technology, as well as for enzyme improvements. The following patents resulted from this project:

- "Rapid detection of mutations in the p53 gene" (5,843,654: filed June 7, 1995, granted December 1, 1998)
- "Rapid detection and identification of pathogens" (6,372,424: filed August 30, 1995, granted April 16, 2002)
- "Detection of nucleic acid sequences by invader-directed cleavage" (5,846,717: filed January 24, 1996, granted December 8, 1998)
- "Invasive cleavage of nucleic acids" (6,348,314: filed July 9, 1996, granted February 19, 2002)
- "Detection of nucleic acid sequences by invader-directed cleavage" (6,001,567: filed July 12, 1996, granted December 14, 1999)

- "Invasive cleavage of nucleic acids" (5,985,557: filed November 26, 1996, granted November 16, 1999)
- "Cleavage of nucleic acid using thermostable *Methanococcus jannaschii* FEN-1 endonucleases" (5,843,669: filed November 29, 1996, granted December 1, 1998)
- "Cleavage of nucleic acids" (6,090,543: filed December 2, 1996, granted July 18, 2000)
- "Cleavage agents" (6,090,606: filed December 2, 1996, granted July 18, 2000)
- "Rapid detection and identification of nucleic acid variants" (5,888,780: filed February 19, 1997, granted March 30, 1999)

Third Wave has published more than 20 papers and has presented more than 30 posters. Third Wave representatives made presentations at 12 conferences during 1995 and 1996 to showcase the information learned from the ATP-funded research. In addition, in 1995 and 1996, various newspapers, magazines, and trade press published six articles about the technology and numerous additional articles have been published since then. The successful ATP project substantially improved Third Wave's credibility, which assisted company executives in obtaining additional capital and in conducting its 2001 initial public offering.

Commercialization Status: By 1997, the ATP-funded research brought the Cleavase system to the point where Third Wave-funded research and development activities could develop products. From 1997 to 2001, Third Wave commercialized several CFLPs.

Outlook: At the close of this project, the commercial outlook for Third Wave's Cleavase technology was uncertain because no products had reached the commercialization phase. Between 1997 and 2001, the outlook improved significantly as numerous products became available. As of January 2002, Third Wave and the entire biotechnology industry have experienced a major downturn, which has led to a significant contraction in the market for the company's products. Third Wave has since reorganized and now focuses on the clinical molecular diagnostic market. Until the current volatility in the biotechnology market ends, the outlook for

Third Wave will be uncertain. However, CFLP-based DNA analysis will continue to be used in laboratories, making the outlook for the technology very good.

Composite Performance Score: * * * *

Number of Employees: Three employees at project start, 195 as of December 2002.

Focused Program: Tools for DNA Diagnostics, 1994

Company:

Third Wave Technologies, Inc.
502 S. Rosa Road
Madison, WI 53719

Contact: Dr. Lance Fors

Phone: (608) 273-8933

Research and data for Status Report 94-05-0012 were collected during December 2001 and December 2002 - January 2003.